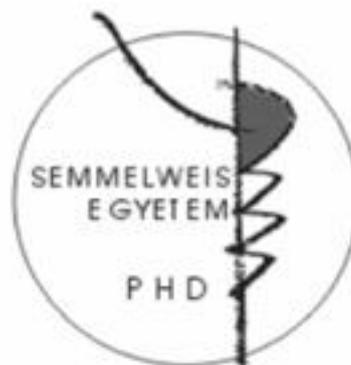


The role of clinical and laboratory markers in the prediction of disease course and response to therapy in inflammatory bowel disease

PhD thesis

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INTRODUCTION

The inflammatory bowel diseases (IBD) e.g. Crohn disease (CD) is a challenge for medicine regarding establishment of diagnosis, the forecast of prognosis, the determination of process and seriousness of disease as well as the forecast of probable consequences of therapy. The procession of Crohn disease cannot give a standardized picture because of a group of patients where the disease exists in more serious form in date of establishment of diagnosis or rather the intergrowth caused by base disease come up earlier. It makes the picture difficult although the localization of disease is relatively constant, the attitude of disease could change during the process.

Inflammatory bowel diseases follows a variable disease course are characterized by periods of remission and relapse. However, disease flare-ups occur in a random way and disease course is often unpredictable. Based on their half-life and changes over time, serum laboratory and fecal markers, alone or in combination, may be used for the prediction of disease activity, as well as short- or medium-term relapses.

C-reactive protein (CRP) is a traditional, non-specific marker of inflammation, and is one of the most important acute phase proteins in humans. The mechanisms leading to CRP elevation in CD are not fully understood. It has been proposed that, in CD patients, the accumulation of mesenteric fat – a major site of IL-6 and TNF- α synthesis – may contribute to CRP production. Another potential explanation is that, during disease flare-up, even in the lack of overt infection, significant bacterial migration occurs due to the transmural inflammation of the gut wall. Bacteremia is one of the strongest stimulators of CRP production.

Crohn's disease is associated with a strong CRP response in both, adult and pediatric populations. CRP is widely used in CD, since it shows acceptable correlation with disease activity, as defined by clinical indices and also with endoscopic or histological activity.

However, although CRP levels in patients with IBD have been measured for many years, several unanswered questions exist regarding the value of this protein as a clinical marker. Only few clinical studies assessed the value of CRP alone or in combination with other, non-specific inflammatory markers in the disease course and outcome in CD patients.

The role of more specific laboratory markers of bacteraemia, however, was rarely assessed. Lipopolysaccharid-binding protein (LBP) plays key roles in promoting innate immunity to Gram-negative bacteria by transferring lipopolysaccharide (LPS) to a binding site of membrane-bound (m)CD14, which represents one part of the cellular LPS-signaling

receptor complex (MD-2/Toll-like receptor 4 [TLR4]). In addition, LBP transfers LPS to soluble (s)CD14, resulting in activation of mCD14-negative cells such as endothelial and epithelial. Along with augmentation of the innate immune response to several bacteria and bacterial surface components, LBP and sCD14 seem to have a more complex immunomodulatory capacity at higher concentrations. Similarly to CRP, LBP is also an early acute-phase protein induced by IL-1, IL-6 and TNF- α . In clinical studies, increased serum LBP concentrations was correlated with the onset of bacteraemia and was a specific and sensitive marker in the differentiation between systemic inflammatory response syndrome (SIRS) and bacterial infection.

One single study assessed the changes in LBP and sCD14 levels in patients with IBD. An increased serum levels of endotoxin, LBP and sCD14 was shown in patients with flare-up and were decreased after treatment. An association between LBP and clinical disease activity indices was also reported. The accuracy of the markers in identifying active disease or predicting an upcoming flare was however not assessed.

The introduction of anti-tumour necrosis factor (anti-TNF) therapies more than 10 years ago and the accumulating evidence from landmark trials and clinical practice has led to a significant change in patient management and treatment algorithms. Anti-TNFs were demonstrated to be effective for the treatment of both luminal and fistulizing disease. Scheduled therapy with both infliximab and later adalimumab was shown to be associated with an increased likelihood of maintaining in remission, achieving mucosal healing in a proportion of the patients, reduced number of hospitalizations and corticosteroid requirements. Adalimumab is a recombinant, fully human, subcutaneously delivered immunoglobulin G1 monoclonal antibody. It was licenced for use in CD in Europe and US in 2007. This anti-TNF agent was found to be effective for treatment of refractory luminal CD, not only in patients who were naive to infliximab but in those who had already been treated with infliximab.

On the other hand anti-TNF trials provided invaluable information how to better optimize the treatment algorithms in patients with CD and raised the threshold for treatment goals. It is now clear that CD treatment must go beyond simply providing symptomatic control and mucosal healing is becoming an increasingly important parameter.

Unfortunately, app. 25%–40% of the patients who respond initially to this therapy need multiple dose and interval adjustments to maintain clinical response over the long-term and about 10% of patients per year discontinue therapy because of loss of response or side effects.

Still relatively little is known on the clinical predictors of maintaining or loss response during adalimumab therapy outside the clinical trials.

In the pivotal clinical trials it has been demonstrated that the anti-TNF agents are more effective in early disease, but the investigations on the importance of concomitant immunosuppressive therapy or biomarkers (e.g. CRP at inclusion) have lead to conflictive results.

Most data are available on the predictors of response for short response and long-term clinical benefit during infliximab therapy. In a recently study by the Leuven group, CD patients who had an elevated baseline levels of CRP ($>3\text{mg/L}$) responded to infliximab therapy better than patients with normal levels. Survival analysis demonstrated that patients who normalized CRP early after 4 of 10 weeks had better long-term benefit. The CRP level at time of endoscopy was significantly correlated with the degree of mucosal healing. Less data are available on the predictors for the efficacy of adalimumab therapy. The best evidence is coming from Belgium, where besides drug trough level, CRP kinetics were identified as a predictor for sustained clinical benefit over a median 20 month adalimumab therapy in CD patients who failed to respond to infliximab. Patients who had normalized CRP levels ($<3\text{mg/L}$) at both week 4 and week 12 discontinued adalimumab less frequently and showed longer sustained clinical benefit. Moreover, time to dose escalation was longer in patients who were treated with immunomodulators.

In addition, combination therapy with immunosuppressants and infliximab has shown to increase efficacy compared to biological only therapy in both azathioprine (AZA) naive and AZA exposed CD patients. In contrast, the importance of concomitant immunosuppression was not proven for adalimumab in post hoc analysis of the randomized controlled trials. Other important clinical variables that have been associated with progressive CD and may influence the also the outcome of the biological therapy include young age-at-onset, steroid dependence (need for repeated steroid courses), structuring/penetrating disease behavior, severe endoscopic lesions and smoking.

AIM OF THE DISSERTATION

Cooperation with home gastroenterology centers during our work process we proposed to **identify potential laboratorial and clinical markers which help to identify patients who have more complicated disease phenotype, process of disease, prospective effect of medication and the prognosis of disease course** in order to identify patients imperiled with evolution of intergrowth as soon as possible and come in for adequate treatment.

Detailed aims:

Serum lipopolysaccharide-binding protein (LBP), and soluble CD14 (sCD14) are markers of disease activity in patients with Crohn`s disease

What is the association between serum LBP and sCD14 and disease activity in CD?

How much is the diagnostic accuracy of LBP, sCD14 and hs-CRP?

What is the association between LBP, sCD14 and disease phenotype in patients with CD?

What is the association between laboratory markers and clinical relapse in CD patients?

High-sensitivity CRP for identification of disease phenotype, active disease, and clinical relapses in Crohn`s disease

What is the role of hs-CRP positivity at diagnosis and accuracy of hs-CRP for identifying active disease during prospective follow-up

What is the association of hs-CRP and clinical relapses relapse during prospective follow-up

What is the association between hs-CRP at diagnosis and disease behavior at diagnosis and medical therapy during follow-up

Predictors of efficacy, mucosal healing and dose intensification during the first year of adalimumab therapy in Hungarian Crohn`s disease

What were the indication of adalimumab therapy and the feature of concomitant medication?

What were the clinical efficacy and predictors of clinical efficacy at 24- and 52-weeks?

What were the predictors of dose intensification?

How much chance of mucosal healing and predictors of mucosal healing?

METHODS

STATISTICAL METHODS

Our data were analysed by courtesy of Péter Varga. We used many statistical program (Statistica 6.0, StatSoft Inc., OK, USA; SPSS13.0, SPSS Inc, Chicago, IL, USA; SPSS15.0, SPSS Inc, Chicago, IL, USA) for analysis. Variables were tested for normality using Shapiro Wilk's W test. χ^2 -test, and χ^2 -test with Yates correction and logistic regression analysis were used to assess the association between categorical clinical variables and clinical/endoscopic outcome. Pearson and Spearman SRO tests were used to analyse the association between continuous variables. Sensitivities, specificities, positive predictive value (PPV) and negative predictive value (NPV) were calculated to determine the predictive power of markers and combination of markers to distinguish between active and inactive CD and clinical relapses. To test the accuracy and to identify cut-off values for markers to identify patients with active disease or clinical relapses, receiver operating characteristics (ROC) curves were generated by plotting sensitivity versus 1-specificity. Kaplan-Meier survival curves were plotted for analysis with LogRank and Breslow tests. Additionally, forward stepwise Coxregression analysis was used to assess the association between categorical clinical variables and probability of clinical relapse. Variables with $p < 0.2$ were included in the multivariate testing. A p value of < 0.05 was considered significant.

DEFINITION OF THE CLINICAL PARAMETERS

The diagnosis was based on the Lennard-Jones Criteria. The disease phenotype (age at onset, duration, location and behaviour) was determined according to the Montreal Classification. Medical records including presence of major extraintestinal manifestations (EIM), previous frequency of flare-ups (frequent flare-up: >1 clinical relapse/year), previous surgery procedures (resections/perianal procedures), the presence of familial IBD, smoking habits, and perianal involvement, were determined by thorough review of the patients' medical charts, which had been collected in a uniform format.

Serum lipopolysaccharide-binding protein (LBP), and soluble CD14 (sCD14) are markers of disease activity in patients with Crohn's disease

Patients

Two-hundred-fourteen well-characterized, unrelated, consecutive CD patients (age: 35.6 ± 13.1 years old, male/female: 95/119, duration: 8.3 ± 7.5 years) were investigated. The

control group consisted of 110 age- and gender-matched healthy blood donors (male/female: 48/62, age: 36.8 ± 12.6 years old). The follow-up period was 12 months in those patients showing no relapse, and until the date of flare in patients with clinical relapse (CDAI>150, Δ CDAI>100 and change in medical therapy), respectively. All relapses were of sufficient severity to warrant a change in treatment.

Detection of LBP and sCD14 and hs-CRP

LBP was determined by a solid-phase enzyme-linked immunosorbent assay based on the sandwich principle (Hycult Biotechnology, Uden, Netherlands). The lower assay sensitivity limit was 1 ng/mL. Soluble CD14 (sCD14) was determined with an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). The lower limits of detection of the assay were 0.125 ng/mL for sCD14. For every sample, 2 analyses on the same plate were carried out and the mean value was used. Duplicate serum samples were taken from a subgroup of CD patients (n=20) both at the time of relapse and after achieving remission (at 12 weeks) to enable to investigate the intra-individual changes of serum LBP and sCD14 during flares, respectively. High-sensitivity C-reactive protein levels were determined in all cases using Integra 700 autoanalyzer system (Roche, Basel, Switzerland).

High-sensitivity CRP for identification of disease phenotype, active disease, and clinical relapses in Crohn's disease

Patients

Two-hundred-sixty well-characterized, unrelated, consecutive CD patients (male/female:120/140, age at presentation: 26.9 ± 11.6 years, disease duration: 7.0 ± 6.1 years) with a complete clinical follow-up were included from two IBD referral centers (Budapest and Debrecen).

Definition of clinical parameters of Crohn disease patients and detection of hs-CRP

Disease activity at the time of the diagnosis and during follow-up visits was calculated according to the Harvey-Bradshaw Index (HBI) index.¹⁸ For the purpose of this study we followed the ECCO guidelines¹⁹ and defined HBI ≤ 4 as a state of remission, 5-7 as mild, and ≥ 8 as moderate to severe disease.

Clinical data and blood samples were prospectively captured between January 1, 2008 and June 1, 2010 either during regular or extraordinary follow-up visits. The follow-up period lasted 12 months in those patients who showed no relapse, or until the date of flare-up in patients with clinical relapse (HBI score >4 , Δ HBI score ≥ 3 and change in medical therapy).

Hs-CRP levels were determined in all cases using Integra 700 autoanalyzer system (Roche, Basel, Switzerland). The lower assay sensitivity limit was 0.1 mg/L, and, the reference range, according to the manufacturer, is 0-5 mg/L.

Predictors of efficacy, mucosal healing and dose intensification during the first year of adalimumab therapy in Hungarian Crohn's disease

Patients

Two-hundred and one well-characterized, unrelated, consecutive CD patients (male/female:112/89, median age at presentation: 24 (IQR: 19-31) years, duration: 8 (4-12) years) with a complete clinical follow-up were included from specialized centers approved for biological therapy in Hungary.

Methods

Clinical, laboratory data and endoscopy results of CD patients in whom adalimumab therapy was started during the first year of the reimbursement period was captured prospectively between December 1, 2008 and December 31 2010. Endoscopic mucosal status was assessed on the basis of the presence of ulcers and erosions. Complete mucosal healing was defined as the absence of any mucosal lesions or signs of active inflammation. A marked improvement of mucosal conditions but still no complete healing was defined as partial healing.

RESULTS

Serum lipopolysaccharide-binding protein (LBP), and soluble CD14 (sCD14) are markers of disease activity in patients with Crohn`s disease

Association between serum LBP and sCD14 and disease activity in CD

Of the 214 CD patients, 65 were in the active, while 149 were in inactive phase of the disease. Mean serum LBP level was significantly higher while sCD14 was lower in both active and inactive disease as compared to the controls (sCD14_{active}= 1784, sCD14_{inactive}= 1361 vs. controls= 2159 ng/mL, p=0.013 and p<0.0001).

In the group of 20 patients who were evaluated both at the time of relapse and afterwards in remission, LBP and sCD14 levels decreased in most patients achieving remission after 12 weeks. Median hs-CRP was also significantly decreased.

There was a significant correlation between LBP, sCD14 and hs-CRP levels in both active and inactive CD by Sperman SRO correlation.

Diagnostic accuracy of LBP, sCD14 and hs-CRP

The accuracy of hs-CRP (AUC= 0.66), sCD14 (AUC= 0.70) was comparable for identifying patients with active disease while the accuracy of LBP (AUC= 0.58) was lower. Cut-off values were calculated from the ROC analysis for LBP and sCD14. The best accuracy for CRP was detected at 11.6 mg/L based on the ROC analysis with a sensitivity of 59% and a specificity of 78.4%.

Association between LBP, sCD14 and disease phenotype in patients with CD

In inactive CD, serum LBP was associated with disease behavior. The highest serum level was detected in CD patients with penetrating form. No other clinically important associations were found between LBP and sCD14 level and disease phenotype in either active or inactive disease.

Association between laboratory markers and clinical relapse in CD patients

From the 91 patients in remission twenty-one patients (23%) relapsed during the 1-year follow-up. The best accurate parameter for the clinical relaps was LBP (OR: 6,5;95% CI: 2,2-19,5, p=0,001). When two markers were positive from three, the relative risk of relapse was 11.8 (95% CI: 3,4-41,2) in univariate analysis.

In Kaplan-Meier analysis, hs-CRP ($p_{\text{LogRank}} = 0.016$, $p_{\text{Breslow}} = 0.019$), LBP ($p_{\text{LogRank}} < 0.001$, $p_{\text{Breslow}} < 0.001$) and sCD14 ($p_{\text{LogRank}} = 0.004$, $p_{\text{Breslow}} = 0.005$) were significantly associated with clinical relapse within 12 months. Moreover, the combination of these markers ($p_{\text{LogRank}} < 0.001$, $p_{\text{Breslow}} < 0.001$) and high relapse frequency in the past ($p_{\text{LogRank}} = 0.036$, $p_{\text{Breslow}} = 0.028$) were significant determinants for the time to clinical relapse.

To further evaluate the effect of the above variables on the probability of clinical relapse, we performed a forward stepwise proportional Cox-regression analysis. LBP, sCD14 and high relapse frequency was independently associated with probability of behavior change.

High-sensitivity CRP for identification of disease phenotype, active disease, and clinical relapses in Crohn's disease

Hs-CRP positivity at diagnosis and accuracy of hs-CRP for identifying active disease during prospective follow-up

32.3% of the CD patients had normal hs-CRP at diagnosis. At the start of the prospective follow-up period (January 1, 2008), the accuracy of hs-CRP to identify patients with active disease was overall good (AUC: 0.82, 95%; CI: 0.77-0.87). The accuracy was better in patients who had elevated hs-CRP at diagnosis (AUC: 0.92, 95%; CI: 0.89-0.97) compared to patients with a normal value at diagnosis (AUC: 0.61, 95%; CI: 0.49-0.73).

The most accurate cut-off value for identifying active disease was 10.7 mg/L in the entire cohort, and 10.3 mg/L in the subgroup of patients with elevated hs-CRP at diagnosis. For internal validation, the ROC analysis was recalculated using the CRP and disease activity data obtained six months after the start of the follow-up period (AUC_{overall}: 0.79, cut-off: 9.7mg/L, AUC_{CRP positive at diagnosis}: 0.87, cut-off: 10.1 mg/L). Therefore, sensitivity analysis was performed using the cut-off value of 10mg/L.

If the cut-off identified by the ROC analysis (3.5 mg/L) was used for the CRP-negative subgroup, the accuracy of the marker to identify active disease later during the prospective follow-up period was better (sensitivity: 55%, specificity: 69%, PPV: 65%, NPV:61%).

Association of hs-CRP and clinical relapses relapse during prospective follow-up

Actual hs-CRP values in patients in clinical remission predicted relapse with reasonable accuracy, both at 3 months (AUC: 0.67, 95%; CI: 0.54-0.80; sensitivity: 65%; specificity:

71%; PPV: 70%; NPV: 67%; cut-off: 10.1mg/L) and 12 months (AUC: 0.63, 95% CI: 0.54-0.73; sensitivity: 52%; specificity: 75%; PPV: 68%; NPV: 62%; cut-off: 8.8mg/L) in the subgroup of patients with an elevated hs-CRP at time of diagnosis. In contrast, the predictive power of hs-CRP to predict clinical relapse during the follow-up period in patients in whom the hs-CRP was not elevated at diagnosis was limited (at 3 months: AUC: 0.53; sensitivity: 0%; specificity: 89%; and at 12 months: AUC: 0.53; sensitivity: 5%; specificity: 87%; PPV: 26%).

In a Kaplan-Meier analysis, hs-CRP was shown to be associated with the probability of 3- and 12-month clinical relapse (at 3 months: $p_{\text{LogRank}}=0.001$, $p_{\text{Breslow}}=0.001$; and at 12 months: $p_{\text{LogRank}}=0.002$, $p_{\text{Breslow}}=0.001$; cut-off: 10mg/L) for patients in clinical remission. The probability of a relapse at 3- and 12 months in the entire cohort was 6.7% and 22% for CRP-negative and 28.5% and 48.4% for CRP-positive patients (absolute $\Delta=15.3\%$ and 19.5%). Accuracy of hs-CRP was better for patients with elevated hs-CRP at diagnosis. The probability of a relapse at 3- and 12 months in this subgroup was 6.9% and 25% for CRP-negative and 24.6 and 51.9% for CRP-positive patients (absolute $\Delta=18.1\%$ and 27.3%). In addition, frequency of relapses ($p_{\text{LogRank}}=0.049$ and $p_{\text{Breslow}}=0.038$) and perianal involvement ($p_{\text{LogRank}}=0.001$ and $p_{\text{Breslow}}=0.001$) was associated with the probability of a clinical relapse within 12-months.

In a proportional Cox-regression analysis, only hs-CRP ($p=0.007$) was independently associated with the probability of clinical relapse at 3-months. In contrast, hs-CRP ($p=0.001$) and perianal involvement ($p=0.01$) were identified as independent predictors for clinical relapse at 12-months. Similarly to the results stated above, hs-CRP was not predictive of clinical relapse at 3- and 12-months in patients without an elevated hs-CRP at time of diagnosis.

Association between hs-CRP at diagnosis and disease behavior at diagnosis and medical therapy during follow-up

Elevated hs-CRP at time of diagnosis was associated with disease location (ileal disease: 43.2%, colonic disease 70%, ileocolonic disease: 72.6%, $p=0.002$), non-inflammatory disease behavior (inflammatory: 36.5%, stenosing/penetrating: 48.9%, $p=0.058$) and need for azathioprine(AZA)/biological therapy during the later course of the disease but not with the need for surgery/reoperation. AZA use in patients with elevated hs-CRP at diagnosis was 81.1% (vs. without 56.5%, $p<0.001$). Biological therapy in patients with elevated hs-CRP at diagnosis was 33.9% (vs. without 20.0%, $p=0.024$).

The association between hs-CRP positivity ($p=0.01$, OR: 1.1, 95%; CI: 1.02-1.23) at diagnosis and subsequent need for AZA therapy remained significant in a logistic regression model adjusted for disease duration (p per year=0.18), location ($p=0.247$), behavior ($p=0.041$, B1: reference; B2: $p=0.73$; B3: $p=0.01$; OR: 3.17, 95%; CI: 1.29-7.79).

Predictors of efficacy, mucosal healing and dose intensification during the first year of adalimumab therapy in Hungarian Crohn's disease

Indication of adalimumab therapy and concomitant medication

Indication for biologic therapy was active luminal disease in 63.7% and active luminal and parallel fistulizing disease in 36.3% of the patients. 97 (48.3%) patients have been treated previously with infliximab therapy. The induction dose was 80/40mg in 61.7%, while 160/80mg in 38.3%. Concomitant immunosuppression at induction therapy was steroids in 41.3%, azathioprine in 69.2% or combined in 26.4% of patients. Hs-CRP at induction was elevated in 66.8% of the patients.

Clinical efficacy and predictors of clinical efficacy at 24- and 52-weeks

Need for concomitant steroids ($p=0.053$)/combined immunosuppression (steroids and azathioprine, $p=0.013$) during induction therapy, previous surgery ($p=0.004$), low CRP at week 12 ($<10\text{mg/L}$, $p<0.001$, $n=189$, patient with a complication before week 12 or a missing week 12 CRP value were excluded) and clinical efficacy (response or remission) at week 4 and week 12 ($p=0.006$ and $p<0.001$) was associated to clinical response or remission at week 24. Clinical response at week 12 ($p<0.001$), low CRP at week 12 ($p<0.001$), previous resective surgery ($p=0.012$) and tendentially need for combined concomitant immunosuppression at induction ($p=0.06$) and higher induction dose of adalimumab (160/80mg, $p=0.065$) were associated with the probability of clinical remission at week 24.

In a logistic regression model, need for combined concomitant immunosuppression ($p=0.022$) at induction, previous operations ($p=0.049$), low CRP at week 12 ($p<0.001$) and clinical efficacy at week 12 ($p<0.001$) were identified as independent predictors for week 24 clinical remission including also induction dose, frequency of relapses, perianal disease and smoking status in the analysis.

At week 52, frequency of previous relapses ($p=0.03$), need for concomitant steroids ($p=0.03$), combined immunosuppression ($p=0.001$), low CRP at week 12 ($<10\text{mg/L}$, $p<0.001$)

and clinical efficacy (response or remission) at week 12 ($p < 0.0001$) was associated to clinical efficacy. Clinical response at week 12 ($p < 0.001$), low CRP at week 12 ($p < 0.001$), need for combined concomitant immunosuppression at induction ($p = 0.018$), frequency of previous relapses ($p = 0.025$) and tendentially smoking ($p = 0.07$) and shorter disease duration (< 3 years; $p = 0.07$) were associated with 52-week clinical remission.

In a logistic regression model, clinical efficacy at week 12 (response or remission) ($p < 0.001$), need for combined concomitant immunosuppression ($p = 0.021$) at induction, short disease duration ($p = 0.03$) and smoking ($p = 0.049$) were identified as independent predictors for 52 week clinical remission. If we included low CRP in the same analysis (and thereby excluded 12 patients from the analysis), results were unchanged, and low CRP at week 12 was identified as an independent predictor.

Gender, location, behavior, perianal disease, presence of extraintestinal manifestations, CRP at the start of the biological therapy, previous anti-TNF therapy or induction dose were not associated to clinical response or remission at either week 24 or week 52.

Need for dose intensification and predictors of dose intensification

Dose intensification to weekly dosing was needed in 16.4% during the one year adalimumab therapy. Parallel azathioprine therapy was inversely associated to escalation to weekly dosing ($p = 0.005$) and in a Kaplan-Meier analysis to time to escalation to weekly dosing ($p_{\text{LogRank}} = 0.003$, $p_{\text{Breslow}} = 0.002$).

Similarly clinical remission at week 12 ($p_{\text{LogRank}} = 0.009$, $p_{\text{Breslow}} = 0.004$) or low CRP at week 12 ($p_{\text{LogRank}} = 0.026$, $p_{\text{Breslow}} = 0.038$) was associated to time to escalation to weekly dosing in a Kaplan-Meier analysis. In a Cox-regression analysis concomitant azathioprine therapy ($p = 0.018$), clinical remission at week 12 ($p = 0.021$) but not CRP ($p = 0.16$) at week 12 was independently associated with the probability of dose escalation.

Mucosal healing and predictors of mucosal healing

Endoscopic partial healing and healing was achieved in 43.1% and 23.6% of CD patients with available endoscopy at week 52 or dropout due to clinical deterioration/surgery ($n = 123$).

Frequency of previous relapses ($p = 0.04$), luminal only disease as indication for treatment ($p = 0.007$), low CRP at week 12 ($p < 0.001$) and being in clinical remission at week 12 ($p = 0.002$) or at week 24 ($p < 0.001$) and tendentially previous IFX therapy ($p = 0.06$) were associated to endoscopic improvement/healing at 12-months. In a logistic regression model, luminal only disease as indication for treatment, low CRP at week 12, being in clinical

remission at week 24, frequency of previous relapses and smoking were associated to endoscopic improvement/healing at 12-months.

CONCLUSIONS

New findings of our investigations are:

1. LBP, sCD14 and hs-CRP are accurate markers of disease activity in CD. Combination of markers add to accuracy of diagnosis.
2. Serum level of LBP, sCD14 and high previous relapse rate were those that independently associated with time to clinical relapse during the subsequent 12-months making these medium term markers of disease course and activity.
3. 10mg/L cut-off value of hs-CRP separate active and inactive Crohn disease patients.
4. High sensitivity-CRP, in patients who were hs-CRP positive at diagnosis, is an accurate marker of disease activity and predictor of short- and medium-term clinical flare-ups during follow-up.
5. In patients who were hs-CRP negative at diagnosis, using of hs-CRP to clinical activity is limited.
6. Elevated hs-CRP at diagnosis was associated with colonic or ileocolonic disease location, non-inflammatory disease behavior and the need for azathioprine or biological therapy during the course of the disease.
7. Serum hs-CRP, in patients with elevated hs- CRP at diagnosis was useful in predicting clinically relevant relapse during prospective follow-up.
8. Hs-CRP was identified as the only independent predictor of clinical relapse at 3-months in Crohn disease patients.
9. Hs-CRP and perianal involvement were identified as independent predictor for 12-months clinical relapse.
10. Clinical efficacy and normalized C-reactive protein at week 12, need for combined immunosuppression, short term disease course and smoking are predictors for medium term clinical efficacy during 12-months adalimumab therapy.
11. Beside adalimumab used azathioprine therapy may decrease the probability for dose escalation.

LIST OF PUBLICATION

Publication directly related

Publications

1. **Kiss LS**, Maria P, Lovasz BD, Vegh Z, Golovics PA, Janka E, Varga E, Szathmari M, Lakatos PL High-sensitivity CRP for identification of disease phenotype, active disease, and clinical relapses in Crohn's disease: a marker for patient classification? *Inflamm Bowel Dis* (accepted in press)
2. **Kiss LS**, Szamosi T, Molnar T, Miheller P, Lakatos L, Vincze A, Palatka K, Barta Z, Gasztonyi B, Salamon A, Horvath G, Tóth GT, Farkas K, Banai J, Tulassay Z, Nagy F, Szenes M, Veres G, Lovasz BA, Vegh Z, Golovics PA, Szathmari M, Papp M, Lakatos PL. Early clinical remission and normalization of CRP are the strongest predictors of efficacy, mucosal healing, and dose escalation during the first year of adalimumab therapy in Crohn's disease. *Aliment Pharmacol Ther* 2011;34:911-22. IF:3.861
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4. **Kiss LS**, Lakatos PL. Natural history of ulcerative colitis; current knowledge. *Current Drug Targets* 2011;12:1390-1395. IF: 3.061
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7. **Kiss LS**, Lakatos PL. A betegség lefolyásának előrejelzése gyulladásoos bélbetegségekben. *Orv Hetil* 2010;151:293-301 (review)
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